The Plight of the Bumble Bee: The Impact of the Bumble Bee Gut Microbiome on Pathogen Infection Rates

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Abstract

Bumble bees are one of the most common and important non-managed pollinators of agricultural crops and are therefore vital to society's ecological and agricultural health. Recent declines in bumble bee populations, likely due to pathogens and land-use change, are concerning and have led to subsequent research on pathogen vulnerability and resistance in bumble bees. Social bumble bees have a specific and relatively consistent gut microbiome. The bumble bee microbiome is typically dominated by only a few bacterial species, and is important for both general and specific resistance against pathogen infections. This gut microbial community has been specifically associated with increased pathogen resistance against the trypanosomatid pathogen *Crithidia bombi,* which is known to increase bumble bee mortality under harsh conditions. Currently little is know about how differences within the microbial community impact rates of infection by *Crithidia*. We examined how differences in the microbiomes of wild and commercial bumble bees may impact *Crithidia* infection rates. Wild and commercial bumble bees were inoculated with microbiomes from both wild and commercial bees, and then infected with *Crithidia*. We found that high OTU diversity, evenness of gut population, and presence of *Apibacter* and *Saccharibacter* in the gut community were all correlated with lower *Crithidia* infection rates. These results indicate that even relatively minor differences between microbial communities can have a significant impact on the microbiome's ability to help protect against pathogen infections.

Introduction

As one of the most common and important non-managed pollinators of agricultural crops, bumble bees are vital to society's ecological and agricultural health. However, bumble bee populations have experienced recent declines, most likely due to pathogens and land-use change

(Cameron et al, 2011). Considering the importance of bumble bees in the agricultural industry, this sudden decrease in wild bumble bee populations is concerning and has subsequently led to research on pathogen vulnerability and resistance in bumble bees. Wild bumble bees have been shown to have a significant impact in the pollination of crops, by pollinating more effectively than managed honey bees. In fact, honey bees have been shown to supplement, but not replace pollination by wild pollinators (Garibaldi et al, 2014). Bumble bees therefore provide vital pollination services to the agricultural industry that cannot simply be substituted by managed honey bees should native bumble bee populations continue to decline.

Like many mammals, both honey bees and bumble bees possess a distinct and specific gut microbiome with the two bee groups sharing similar bacterial taxa (Mohr & Tebbe 2006; Koch & Schmid-Hempel 2011a; Martinson et al. 2011). Although the gut microbiome of honey bees and bumble bees is simpler than that observed in mammals, including humans (Martinson et al. 2011), it nonetheless shares several features with the more complex microbiomes of mammals. Both bumble bee and mammalian microbiomes are socially transmitted (Koch et al. 2013), and, as has been found in humans (Schloissnig et al. 2013), the composition of bumble bee gut microbiomes differs slightly among individual hosts. The similarities between the simple gut microbiomes of bumble bees and more complex microbiomes allow the bumble bee microbiome to serve as a model system for studying symbiotic relationships in the gut (Li et al, 2015).

Compared to solitary bee species, the social bumble bees have specific and relatively consistent gut microbiome (Martinson et al. 2011). The bumble bee microbiome is typically dominated by only a few bacterial species, including *Gilliamella apicola*, *Snodgrassella alvi*, and several species of *Lactobacillus* (Martinson et al, 2011; Koch & Schmid-Hempel 2011b). This

microbial composition is important for both general and specific resistance against pathogen infections (Koch & Schmid-Hempel, 2012). Specifically, the gut microbial community has been hypothesized to complement the host immune system by helping defend against parasites that enter the bumble bee through the gut (Koch & Schmid-Hempel, 2012).

One such parasite, *Crithidia bombi*, is a trypanosomatid commonly found in bumble bees (Salute et al, 2011). *Crithidia* has been shown to increase bumble bee mortality under harsh conditions (Brown et al, 2000), and reduce colony founding success of infected queens by 40%, while also leading to significant reductions in mass among infected queens, reductions in colony size, male production, and overall fitness (Brown et al, 2003). However, the presence of a gut microbiome has been shown to significantly decrease the infection rates of *Crithidia bombi* (Koch & Schmid-Hempel, 2011).

Bumble bees have been bred for use as commercial pollinators since 1988 (Inari et al, 2008). These bumble bee pollinators have been used to supplement pollination by honey bees, and as a consequence of recent honey bee declines, have become the sole pollinators of certain crops (Owen et al, 2016). While both captive bred and wild bumble bees have been used in research, the resident microbiomes of captive versus wild bumble bees have rarely been directly compared (Meeus et al. 2015; Parmentier et al. 2015). Understanding how such environmental circumstances affects the gut microbiome and, consequently, the health of the host, could have important implications. It has been demonstrated that captive bred bumble bees often have higher pathogen infection rates than wild bumble bees, and can then spread those diseases to wild bumble bee populations (Manley et al, 2015). Hence, prevalent pathogen infections among captive bred bumble bees could be a contributing factor in the decline of wild bumble bee populations.

Bombus impatiens is a widespread commercial bee species, endemic to North America, and is the sole species sold commercially across North America for pollination services. It is a highly successful and readily available pollinator for a variety of greenhouse crops, including tomatos (Kevan et al, 1990), muskmelons (Fisher and Pomeroy, 1989), and sweet peppers (Shipp et al. 1994), while also showing potential for effective pollination of field crops (Stanghellini et al, 1997). We hypothesize that captive breeding over many generations in *Bombus impatiens* has altered the bacterial microbiome (due to factors such as an artificial environment, antimicrobial food, or transmission bottlenecks). This may have led to the loss of overall microbiome diversity and/or of specific bacterial strains. These changes in the microbiome may have made domesticated bees more susceptible to disease.

This study investigates how differences in the gut microbiomes of captive and wild bumble bees impact their ability to fight off pathogen infection. This research will test the role of the microbiome and host genotype in pathogen susceptibility of domesticated and wild *B. impatiens*. Because of the recent declines in bumble bee populations, information on how variations in the bumble bee microbiome may affect pathogen susceptibility could inform future research on pathogen infection rates among bumble bees. Furthermore, it could serve as a basis for future experiments seeking to improve the ability of bumble bees to defend themselves against infection by common pathogens.

Methods

Bumble Bee Colonies

Wild *B. impatiens* queens were collected in east Texas in April of 2015, after emerging from winter hibernation. The queens were kept in individual cages in a laboratory incubator, which was kept at 28 °C, 60% humidity. The queens were fed a diet of pollen and sucrose water

(1:1 w/v), and allowed to start a colony. Commercial bumble bees were ordered from BioBest (Westerlo, Belgium) and kept in the lab on a diet of pollen (Betterbee irradiated pollen) and sucrose water.

Experimental Procedure

Preparation of Treatment Groups

Worker *B. impatiens* from both wild and commercial colonies were inoculated with one of five microbiome treatments. Wild caught queens and commercial queens were used to generate five categories of microbiomes: wild-single, wild-combination, commercial-single, commercial-combination, and filtrate. In order to generate the wild-single microbiome treatment, one whole gut was removed from a wild queen, and 10 mM phosphate buffered saline (PBS) was added so that the total volume was 250 µL. The gut was then homogenized, and 100 µL was set aside for the filtrate treatment. 150 µL of glycerol were then added to the remaining 150 µL, resulting in a total volume of 300 µL at 15% v/v glycerol. This mixture was then separated into 60 five µL aliquots and frozen at -80°C.

In order to generate the wild-combination treatment, whole guts were removed from four wild queens. Sufficient PBS was added in order to bring the total volume to 1000 µL, and 400 µL were set aside for the filtrate treatment. The remaining 600 µL were mixed with a glycerol solution to yield 1200 µL at 15% v/v glycerol. The mixture was then separated into 5 mL aliquots and frozen at -80°C.

The above process was followed in order to generate the commercial-single and commercial-combination microbiome treatments. The filtrate treatment was generated from the set aside homogenized guts from each treatment. The guts were filtered through 4 layers of cheese cloth and then brought to a 15% v/v glycerol concentration. This method of filtration was

determined to be sufficient to remove the majority of relevant gut bacteria, while leaving other gut (nonbacterial) elements that may affect pathogen infectivity. 75 µL from each treatment group was then combined to form the filtrate treatment group, which was divided into 5 µL aliquots and frozen at -80°C.

Inoculation

In order to generate germ-free bumble bees, *B. impatiens* worker cocoons were removed from the wild and commercial colonies. Four wild colonies and six commercial colonies were used for the experiment. The pupae were removed from the cocoon and allowed to mature in sterile conditions at 28 °C, 60% humidity. Upon reaching maturity, they were transferred to individual sterile plastic cages and inoculated with a treatment group. Bees from each colony were assigned random treatment groups. The prepared treatment aliquot was allowed to thaw on ice and then combined with 10 mL of filtered sugar water for a 2:1 ratio of sucrose water to inoculum. This mixture was then fed to bees that had been starved for 3 to 5 hours. The bees were monitored to ensure that they consumed the inoculum. Following the inoculation, workers were fed filter-sterilized sucrose water and gamma-irradiated pollen *ad libitum*.

Infection

After allowing seven days for the administered microbiome to establish itself in the gut, bees were removed from their cup cages and starved for 3 to 5 hours. The *Crithidia bombi* strain used for infection was isolated from bumble bees collected in New Jersey. *Crithidia* was grown in Insectagro media supplemented with 5% FBS at 28 ^{o}C, 3 % CO_2. The concentration of the *Crithidia* culture was determined using a counting chamber, and then a solution was prepared so that the bees were fed 5 µL of *Crithidia* culture, containing 15,000 cells of *Crithidia*. This was

then combined with 10 mL of filtered sugar water and fed to the bumble bee. The bees were monitored to ensure that they consumed the infection mixture.

Pathogen Infection Count

After another seven days, shown to be within the peak period of *Crithidia* infection (Schmid-Hempel & Schmid-Hempel, 1993), the bees were placed on ice and dissected. The gut was removed and homogenized in 200 mL PBS. The level of *Crithidia* infection was recorded by counting the number of *Crithidia* cells using a counting chamber. The remaining homogenized gut and bee carcass were stored at -80°C.

Evaluation of Bumble Bee Microbiomes

Five individual guts from each of the ten treatment groups were randomly selected for DNA extraction (according to the protocol of Engel et al. 2013). After DNA extraction, the DNA concentration for each of these 50 samples were determined using a Qubit 3.0 Fluorometer (Thermo Fisher Scientific Inc.). Samples were diluted to 10 ng/μL and sent to the University of Texas Austin Genomic Sequencing and Analysis Facility, where the V4 region of the bacterial 16S rRNA gene was amplified by PCR using universal bacterial primers. This avoided amplifying any eukaryotic bumble bee DNA, while amplifying bacterial DNA. Amplicon libraries for each samples were then prepared and sequenced on the Illumina MiSeq platform (Illumina Inc.) with a 2 × 250 bp paired read design.

Statistical Analyses

Infection load was measured as the number of *Crithidia bombi* cells present in 10 uL of homogenized gut, as estimated using a cell counting chamber. The *Crithidia* count data was log-transformed and a two-way ANOVA was run using the microbiome treatment and the bee type (wild or commercial) as factors. Analyses were done in R version 3.2.3 (R Core Team, 2015).

Bioinformative analysis of 16S rDNA sequences

Sequences were processed and analyzed using QIIME v1.9.1 (Caporaso et al. 2010). Raw sequence reads were trimmed so that primer sequences were removed from the reads. Reads were then filtered for quality and sequence length, where the maximum unacceptable Phred quality score was Q29, sequences with ambiguous or unassigned characters were excluded, and the minimum number of consecutive low quality based cells required to include a read was 0.8 of the input read length. The minimum sequence length was set at 230 nucleotides, and the maximum sequence length was set at 270 nucleotides. Sequences were then clustered into operational taxonomic until (OTUs) at 97% sequence similarity, since 97% is appropriate for species-level phylotypes (Caporaso et al, 2010). Representative sequences were then chosen for each OTU. Any OTUs accounting for less than 0.5% of the reads for that sample were removed from the analysis, since they could result from multiplexing barcode assignment errors. OTU identities were then determined using BLAST, and OTUs were compiled into an OTU count table with taxonomy. The OTU table underwent further filtering to remove plastid and mitochondrial DNA reads and non-bacterial reads. Samples with OTU counts lower than 500 were also removed from the analysis. The OTU table was then used to perform alpha and beta diversity calculations. Beta diversity analyzes similarity between samples, and the results were visualized on PCoA cluster plots. Two methods of analysis were used, Bray Curtis and Binary Jaccard. Binary Jaccard analyzes similarity by considering only the presence versus absence of OTUs, while Bray Curtis considers the presence versus absence as well as abundance of OTUs. Both of these were statistically analyzed for significant clustering using ANOSIM.

Results

Crithidia Infection

Crithidia infection levels were compared between species and treatment groups (Figure 1). With a two-way ANOVA I found significant main effects of microbiome ($F(4,138)= 18.6$, $p<0.01$) and bee type ($F(1,138)=12.0$, $p<0.01$). However, there was not a significant interaction between bee type and microbiome ($F(4,138)= 0.967$, $p>0.05$). A Tukey's pairwise comparison revealed significant differences between the commercial-combination treatment and the wild-single treatment ($p< 0.01$), between the commercial-single and wild-single treatments ($p<0.01$), between wild-combination and wild-single treatments ($p<0.01$), between filtrate and wild-single treatments ($p<0.01$), and between and filtrate and commercial-combination treatments ($p<0.01$). No significant differences were found between the commercial-single and commercial combination treatments ($p>0.05$), wild-combination and commercial-combination treatments ($p>0.05$), wild-combination and commercial-single treatments ($p>0.05$), filtrate and commercial single treatments ($p>0.05$), or between filtrate and wild-combination treatments ($p>0.05$).

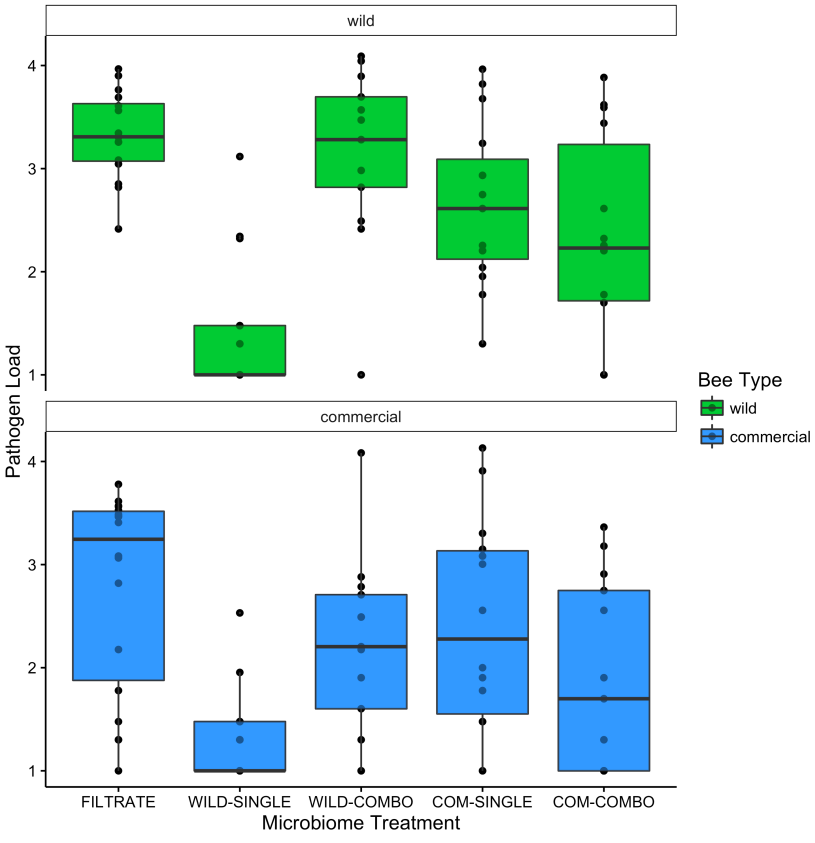


Figure 1. Results of two-way ANOVA. Box plot shows comparisons of effectiveness of each treatment group (Filtrate, Wild-single, Wild-combo, Com-single, Com-combo) in reducing *Crithidia bombi* infection loads. Infection loads are compared between treatment groups and also between wild and commercial bees. The treatment groups wild-single and com-combo were the only treatment that resulted in a significantly lower infection load than the Filtrate treatment group.

Table 1: Summary of Reads from Bumble bee Samples	
Bacterial 16S rRNA reads	2,190,342
Average # bacterial sequences per bee	47,616 (range 146–87397)
97% id clusters	4009
97% clusters contributing > 0.5% of reads in any sample	39
# reads in these 39 clusters	2,121,512
Bacterial sequences in known bee phylotypes	
Apibacter	6.3%
Bifidobacterium	0.3%
Bombiscardovia	0.1%
Lactobacillus (Firm 4 and Firm 5)	14.3%
Gilliamella	45.3%
Saccharibacter	1.4%
Snodgrassella	13.3%
Other observed sequences	
Microbacterium	< 0.1%
Staphylococcus	0.9%
Crithidia bombi	17.1%

Bacterial Sequencing Results

Following quality control, a total of 2,190,342 bacterial sequences were retrieved, and these formed 4009 clusters of 97% or greater sequence identity (OTU_{97}). Of these, only 39 constituted at least 0.5% of sequences in any single bee. For 8 of these, the top blastn hits corresponded to sequences for phylotypes previously sampled from bees. There was also one sample with greater than 0.5% of *Staphylococcus*, one sample with greater than 0.5% of *Microbacterium* and several samples with greater than 0.5% of *Crithidia*. Seven typical bee phylotypes were represented by a single OTU_{97} (*Snodgrassella*, *Lactobacillus Firm-4*, *Lactobacillus Firm-5*, *Apibacter*, *Bifidobacterium*, *Bombiscardovia*, *Saccharibacter*) and one

typical bee phylotype was represented by six $OTUs_{97}$ (*Gilliamella*).

Alpha diversity analysis at read depth 550 was used to analyze the collected data and generate plots of Shannon's H diversity and observed OTUs (Figure 2). The wild-single treatment group was statistically more diverse than any of the other treatment groups (Shannon's H, $F(4,34)=4.96$; $p<0.01$). The wild-single treatment also had significantly higher observed OTU numbers than the other treatment groups ($F(4,34)=8.39$; $p<0.01$).

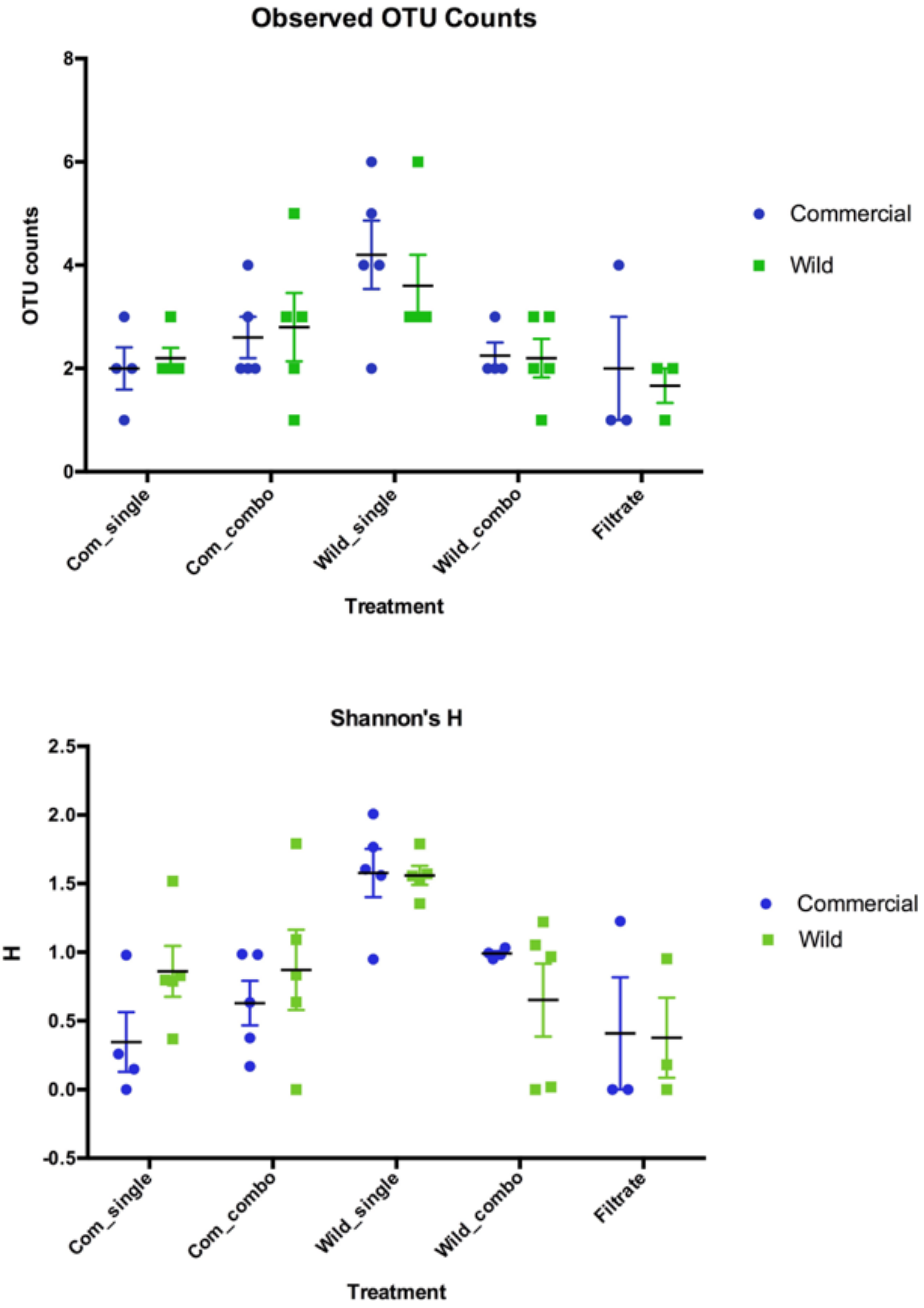


Figure 2. Results of two-way ANOVA of alpha-diversity measurements. A) Observed OTU counts are compared between the five treatment groups. The wild-single treatment group has significantly higher observed OTU counts than the other treatment groups. B) Shannon's H, measuring evenness of representation in the OTU population, is compared across the five treatment groups. The wild-single treatment group had a significantly higher H value than the other treatment groups.

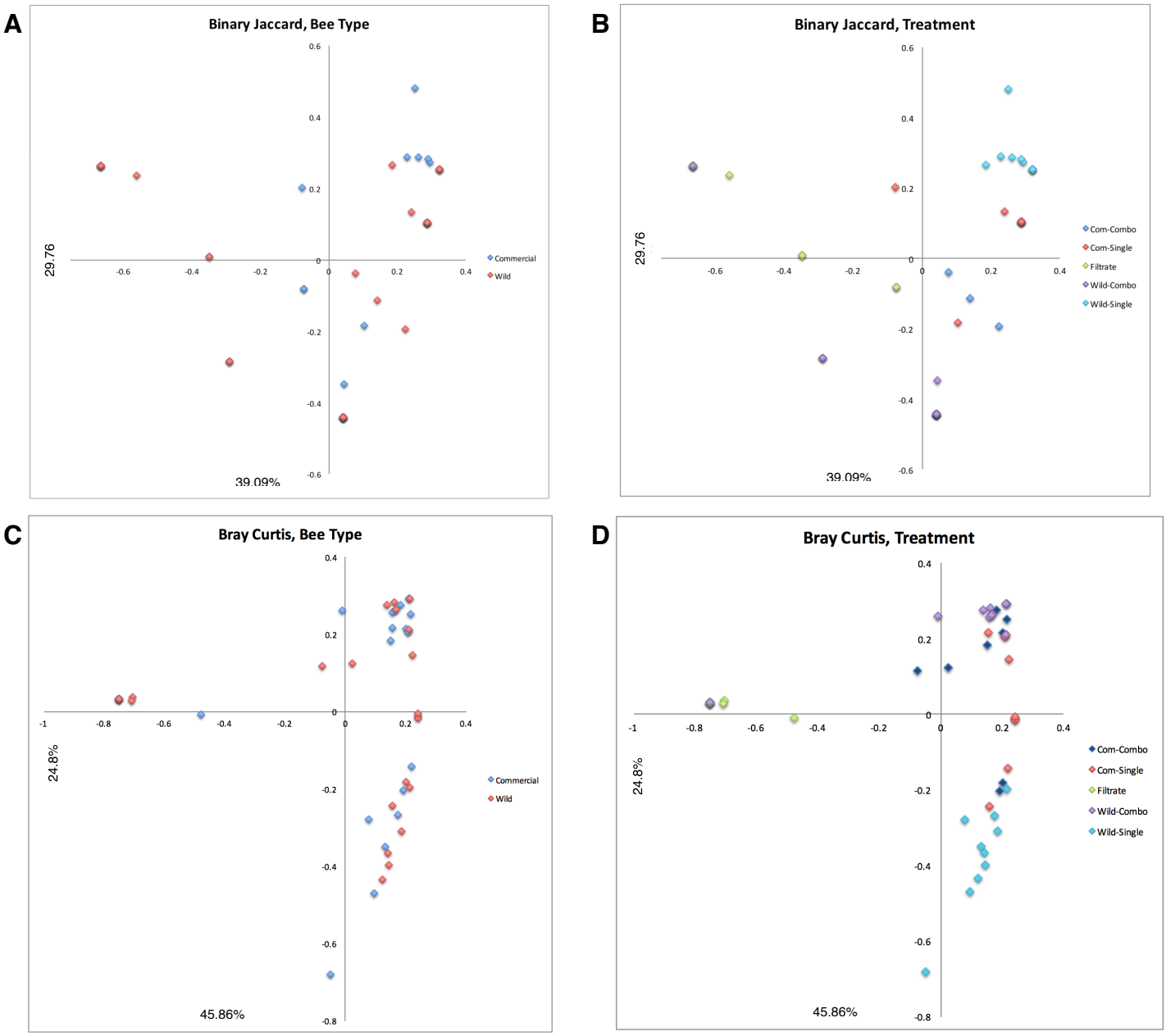
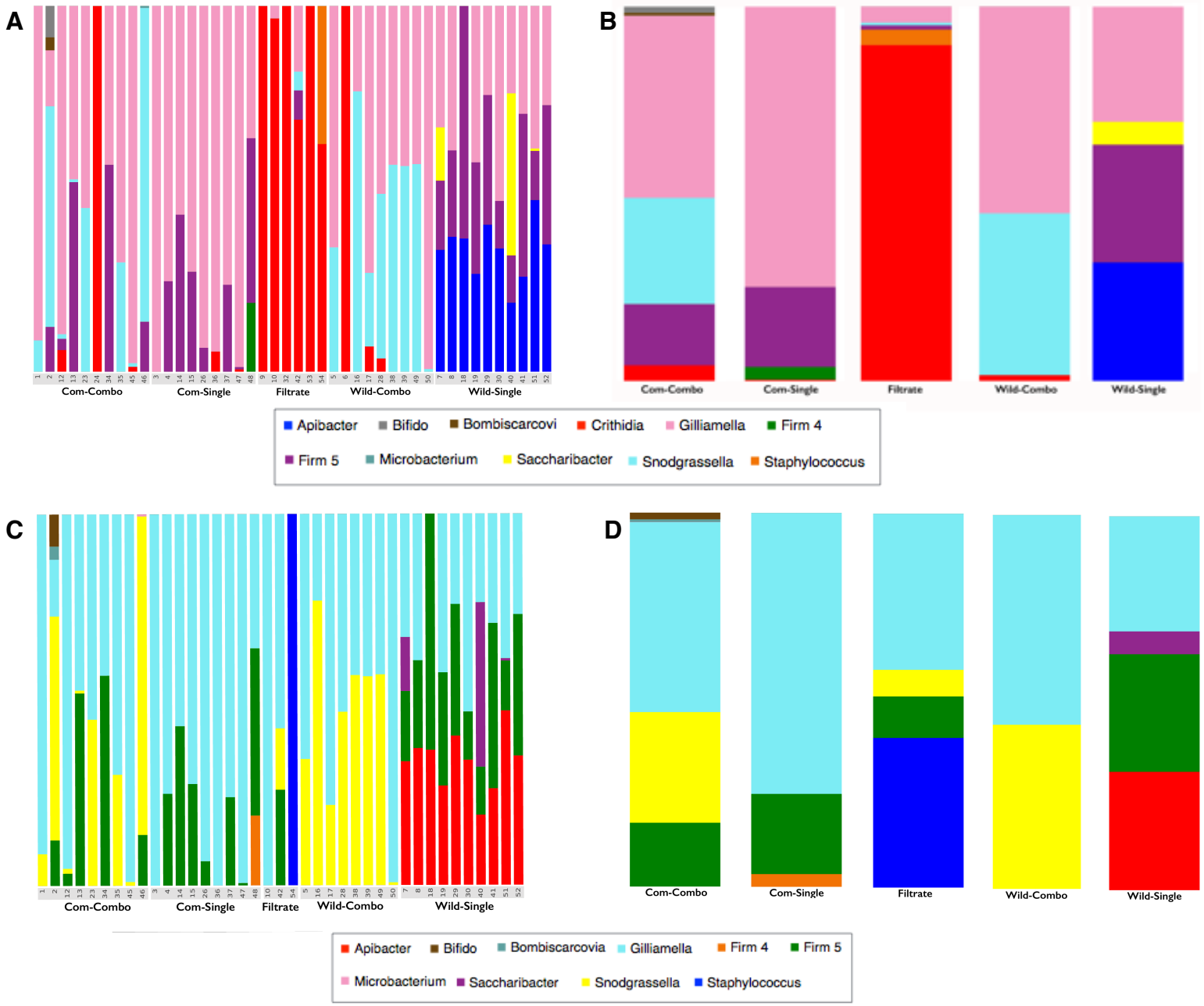


Figure 3. Results of (PCoA) performed on bacterial gut communities. A) Clustering based on bee type (wild or commercial), not abundance weighted. The x-axis explains 39.09% of the variation and the y-axis explains 29.76% of the variation in the gut communities. B) Clustering based on treatment, not abundance weighted. The x-axis explains 39.09% of the variation and the y-axis explains 29.76% of the variation in the gut communities. C) Clustering based on bee type, abundance weighted. The x-axis explains 45.86% of the variation and the y-axis explains 24.8% of the variation in the gut communities. D) Clustering based on treatment, abundance weighted. The x-axis explains 45.86% of the variation and the y-axis explains 24.8% of the variation in the gut communities.

Beta diversity analysis at read depth 550 indicated that the samples clustered most strongly by treatment group, with the Wild-Single treatment group clustering most closely, indicating that their gut microbial communities are more similar to each other than to bees in the other treatment groups. Samples did not cluster based on bee type (wild or commercial), nor for colony of origin (Figure 3), indicating that there is not a significant effect of host background on microbial community structure. Statistical analysis of beta diversity via ANOSIM showed that microbial community composition did differ among treatment groups (ANOSIM $F(5,44) = 0.58$; $P<0.001$). Further analysis also showed that microbial community composition was not statistically different between bee types (ANOSIM $F(2,44)=-0.02989$; $p>0.05$), or between colonies of origin (ANOSIM $F(10,44)=0.0199$; $p>0.05$).

Qualitative assessment of the relative abundances of OTUs revealed that there were substantial differences in the bacterial compositions of the five treatment groups (Figure 4). The wild-single treatment group had a microbiome that was distinct from the other treatment groups, on average consisting of 30.78% *Gilliamella*, 6.11% *Saccharibacter*, 31.44% *Lactobacillus* (Firm 5), and 31.66% *Apibacter*. None of the other treatment groups had detectable levels of *Saccharibacter* or *Apibacter*. The control (filtrate) group had low percentages of typical gut bacteria and was largely populated by *Crithidia* (Figure 4; A, B). When *Crithidia* was removed from the analysis, several of the filtrate samples no longer contained any assigned OTUs (samples 9, 32, and 53). This indicates that the control treatment was successful in producing bees with little or no gut bacteria, such that PCR of 16S rRNA was only able to amplify *Crithidia* sequences instead of bacteria. Other samples that had no bacterial OTUs were sample 24 from com-combo, and sample 6 from wild-combo. The resulting figure reinforced the analysis in which *Crithidia* was included, while providing more detailed information about the



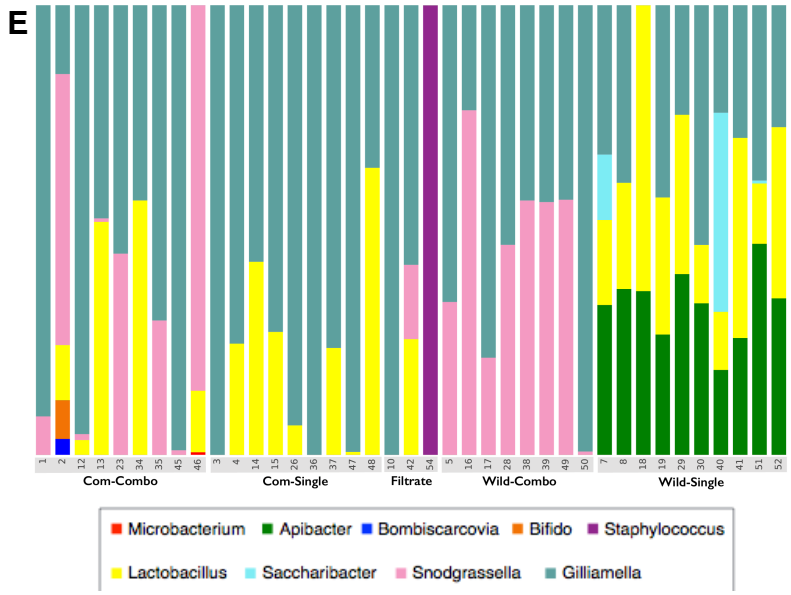


Figure 4. Summary of microbial communities in bumble bee samples ordered by taxonomy. A) Microbial composition of individual samples, ordered by treatment group. *Crithidia* sequences are included and *Lactobacillus* bacteria are labelled separately as Firm 4 and Firm 5. B) Microbial composition of treatment groups, *Crithidia* sequences are included and *Lactobacillus* bacteria are labelled separately. C) Microbial composition of individual samples, *Crithidia* sequences are not included and *Lactobacillus* bacteria are labelled separately as Firm 4 and Firm 5. D) Microbial composition of treatment groups, *Crithidia* sequences are not included and and *Lactobacillus* bacteria are labelled separately. E) Microbial composition of individual samples, ordered by treatment group, *Crithidia* sequences are not included and Lactobacillus bacteria are not labelled *separately*.

samples that were mainly composed of *Crithidia*. None of these samples contained *Apibacter* or *Saccharibacter* (Figure 4; B, C). Furthermore, the wild-single samples on average contained a more complex and evenly distributed microbial composition.

The samples used in this study were also compared to bees collected from the wild in New Jersey. Guts removed from the wild bumble bees were included in the analysis for Figure 5, shown below. The wild-collected microbiomes serve as a background for the wild-single and wild-combo treatments. Neither of the wild-treatment groups accurately mimic the gut of collected wild bumble bees. However, the wild-single treatment group does have relatively similar proportions of the main observed bacteria (Wild-Single: 31.44% *Gilliamella*, 6.06% *Saccharibacter*, 31.15% *Lactobacillus*, 31.36% *Apibacter*. Collected Wild: 30.08% *Gilliamella*, 14.15% *Snodgrassella*, 2.89% *Saccharibacter*, 18.55% *Lactobacillus*, 2.56% *Apibacter*). However, the wild collected bee guts had a greater number of OTUs present, including several environmental bacteria.

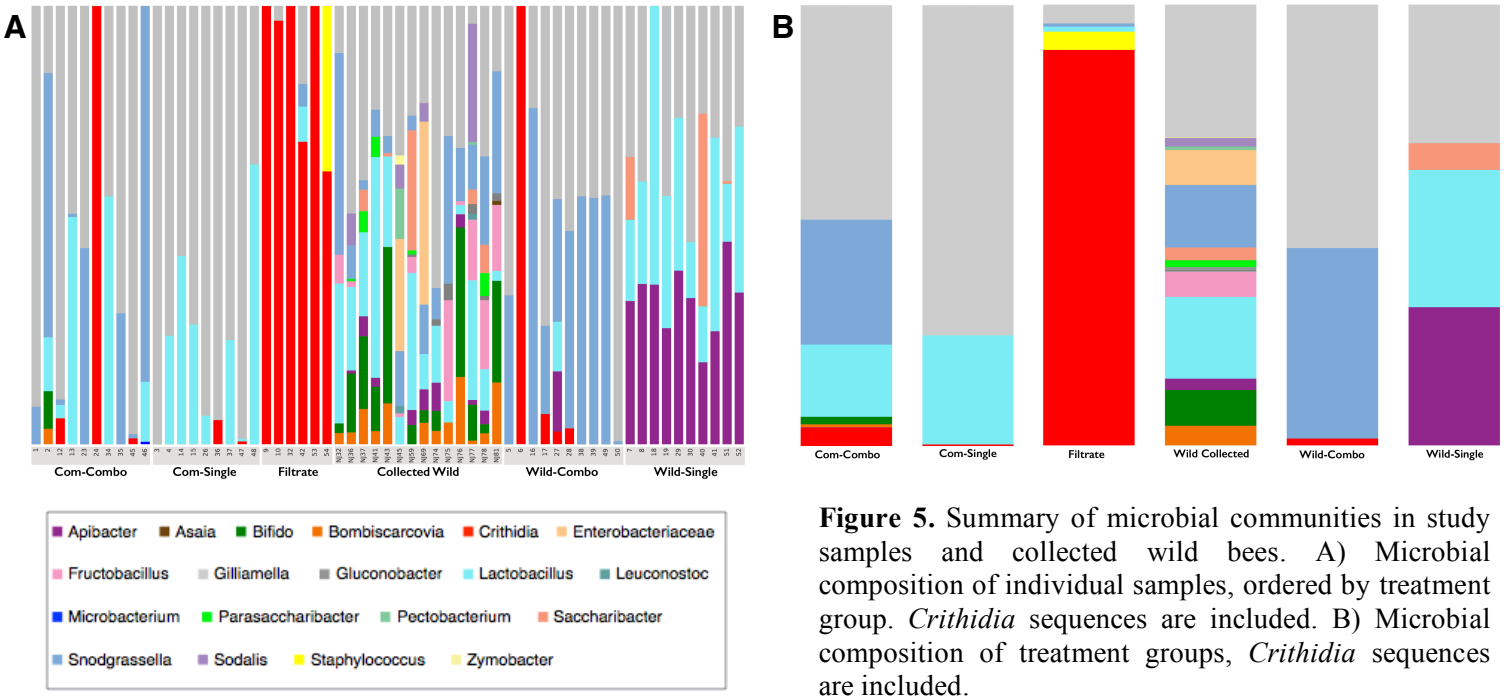


Figure 5. Summary of microbial communities in study samples and collected wild bees. A) Microbial composition of individual samples, ordered by treatment group. *Crithidia* sequences are included. B) Microbial composition of treatment groups, *Crithidia* sequences are included.

Discussion

Of the five treatment groups, only wild-single microbiome and the com-combo treatments resulted in a significant difference between the resulting *Crithidia* infection load and that of the filtrate or control treatment. However, the com-combo treatment had a smaller effect on the decrease of the pathogen load. Furthermore, although significantly different than the control group, the pathogen load resulting from the com-combo treatment was not significantly different from the non-significant treatment groups (wild-combo, com-single). There was also a significant impact by the two levels of the experiment, bee type and bee microbiome, where the commercial bees appear to generally have lower *Crithidia* infection loads than wild bees. However, the observed impact of the microbiome was more significant. Analysis of sample microbiomes did not reveal significant differences between wild and commercial bees in their acquisition of our treatment microbiomes (see Figure 3). It is likely that the lower *Crithidia* infection levels observed in commercial bees are not entirely due to factors in the bee microbiome. This is interesting as it might indicate that commercial bumble bees have significant mechanisms outside of the microbiome that decrease *Crithidia* infection levels, which are not observed in wild bumble bees.

Further analysis of the five treatment groups revealed that, in addition to producing significantly lower *Crithidia* infection loads, the wild-single microbiome was also significantly different in its microbial composition from the other treatment groups. Statistical analysis of alpha diversity showed that the wild single group had significantly higher numbers of gut OTUs than the other treatment groups. It also had significantly higher Shannon's H values, indicating that the wild-single treatment was more diverse, while the lower Shannon's H values of the other treatment groups indicate that one or more OTUs dominated the gut populations of those groups.

The PCoA plots also show significant clustering of the wild-single treatment. It is interesting that the com-combo treatment group resulted in lower infection rates, but did not have the same significant high observed OTU or Shannon's H values. Furthermore, the com-combo samples only appear to cluster on the PCoA plots when abundance weighted, but not otherwise. This may indicate that additional com-combo samples should be sequenced in order to understand why this treatment group significantly reduced *Crithidia* infection levels compared to the control group.

As mentioned above, a significant difference between infection levels of the two bee types is not observed when the microbiomes of the two bee types are compared (Figure 3;A,C). This indicates that the microbiome treatments were not significantly different when applied to the two bee types. Further research will be required to determine if, when infected with equal amounts of *Crithidia*, commercial bees have significantly lower infection loads than wild bumble bees. Based on the data collected for this study, it appears that if it exists, this difference is not caused by variations in the gut microbiome, but by alternative and currently unknown mechanisms.

Also of interest is the fact that the wild-combo treatment failed to reduce *Crithidia* infection loads, while the wild-single treatment significantly decreased the level of *Crithidia* infection in both the wild and commercial bees. When the composition of the two guts are compared, there are apparent differences in the microbial communities of the two treatments. The wild-single treatment group had significantly higher numbers of observed OTUs, and the bacterial population of the wild-single treatment group was proportionally more even than the wild-combo treatment group. Additionally, there were distinct differences between the composition of the wild-single group compared to the wild-combo group. The wild-single group had distinct OTUs that were largely absent in the the wild-combo group, specifically *Apibacter*, *Lactobacillus*, and *Saccharibacter* (Figure 4). It is possible that the wild bee used to prepare the

wild-single treatment had a non-representative microbiome. It is also possible that combining the four wild microbiomes to prepare the wild-combo treatment in some way altered the composition of the resulting treatment group. An alternative possibility is that the combination of distinct microbiomes were unable to fully propagate in the guts of the treatment bees, leaving only *Snodgrassella* and *Gilliamella*. Sequencing the treatment group microbial communities could provide further information on whether there were additional bacteria present in the treatment group that were unable to succeed in the bumble bee gut, or whether the initial treatment group contained only the bacteria we see in the wild-combo bumble bees.

In addition to the higher numbers of observed OTUs and a more even OTU representation in the wild-single microbiome, there were also differences in the actual bacteria present in the wild-single treatment group and the other treatment groups. While the com-combo, wild-combo, and filtrate groups contained *Gilliamella* and *Snodgrassella*, neither the wild-single nor the com-single treatment groups had *Snodgrassella* present in their guts. The com-single treatment group only contained *Lactobacillus* and *Gilliamella*, while the wild-single group had *Apibachter*, *Gilliamella*, *Lactobacillus*, and *Saccharibacter*, but no *Snodgrassella*. This is interesting considering the success of the wild-single treatment group in reducing the *Crithidia* infection load and previous research showing that gut colonization by *Gilliamella* was associated with decreased *Crithidia* infection levels (Koch and Schmid-Hempel 2011; Cariveau et al, 2014). Furthermore, the only bacteria that the wild-single treatment group had and the other lacked were *Apibacter* and *Saccharibacter*. It is possible that one or both of these two bacteria provide additional protection against infection by the pathogen *Crithidia*. However, additional research will need to be done in order to more fully determine the importance of these bacteria in bumble bee health.

Of further interest is the difference in the bacterial populations present in the wild treatments groups and the wild collected bees. Neither wild treatment group closely resembles the bacterial population present in the wild collected bees. The wild collected microbiomes had a greater number of OTUs present than both wild treatment groups, including several plant-associated or environmental bacteria. The bees given the treatment microbiome were fed a diet of irradiated pollen and sugar water, so it is not surprising that they lack the diversity of environmental bacteria observed in the wild collected bumble bees. Also, the wild microbiome in treatments was collected in a different time and place (East Texas) from the collected wild bees (New Jersey), and these wild bee populations may have different microbiota composition. Recent studies have shown that bumble bees reared indoors have a relatively standard core set of bacteria, but lack the non-core bacteria that are present and variable in wild bumble bees (Meeus et al, 2015). However, we do not know how these environmental bacteria might impact a bumble bee's ability to fight off an infection by *Crithidia*. It would be interesting to see if the greater bacterial diversity and higher number of OTUs resulted in even lower *Crithidia* infection rates than the wild-single treatment group did. This difference in microbiomes between lab bees and wild bees also highlights the complications of applying research to wild bee populations.

Understanding the health of bumble bees is vital to the continued success of our agricultural industry. Bumble bees are the most common and important non-managed pollinators, and are also specifically the most important pollinators for many agricultural crops as well as wild plants (Garibaldi et al, 2013). My research indicates that there may be multiple aspects of gut microbiome composition influencing the health of bumble bees. High OTU diversity, evenness of gut population, and presence of *Apibacter* and *Saccharibacter* were all correlated with lower *Crithidia* infection rates.

Future research should focus on several aspects of this project. Each factor that was correlated with lower *Crithidia* infection should be individually investigated in relation to its impact on *Crithidia* resistance. Specifically, *Apibachter* and *Saccharibacter* should be investigated to see if by themselves they significantly decrease *Crithidia* infection levels. Previously only *Gilliamella* and *Snodgrassella* have been correlated with *Crithidia* resistance, so the addition of *Apibacter* and *Saccharibacter* to this group would be a novel advance.

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